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BIOCHEMICAL DIFFERENCES IN NORMAL HUMAN AND DOWN'S SYNDROME FIBROBLASTS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Biochemical Differences in Human Normal and Down's syndrome Fibroblasts" submitted by Alfonso Iafolla in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

Biochemical differences in human mongoloid (Down's syndrome) fibroblast cultures and normal human fibroblasts were compared. Differences in the phases of the cell cycle were elucidated by the synchronous and asynchronous labelling with tritiated Thymidine. D.S. cells have a longer S period compared to normals. Continuous labelling experiments with synchronous cultures demonstrated a 2 - 4 hour longer S phase and a 1 - 2 hour shorter G_1 in D.S. Generation times of mongoloid cells were shown to be 20 - 22 hours compared to 14 - 15 hours for comparable aged normal cells.

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INTRODUCTION AND LITERATURE REVIEW

A cell cycle may be defined as the interval between two successive mitoses. In a 1953 publication, Howard and Pelc[23] implied that DNA was synthesized during the intermitotic period. They designated the phases of the cell cycle as G_1, G_2, M and S. Mitosis (M) is followed by a quiet metabolic phase (G_1). In mid cycle DNA is synthesized (S). This in turn is succeeded by a period of constructive activity (G_2) in preparation for the next mitosis (M).

Many papers have appeared since 1953 which delineate the biochemistry of continuously dividing cell lines. Less has been published on cell strains which have a finite life span. Cells lines divide indefinitely for an indeterminate period. Baserga[45] has elaborated the concept of the G_o state in which the cells are quiescent. That is, there is no evidence of DNA or RNA replication or turnover. The cell strains in G differentiate and synthesize protein, however, nucleic acid metabolism stops[3,6,28]. Contact inhibition of growth arrests the human cells in the G phase of the cell cycle[4,6,38]. This property of G_o cells was used to induce synchronous division. G_1 is the pre-DNA synthesis resting state so G_o can be regarded as an indefinitely prolonged G_1.

Various reports conflict on the generation time of human diploid cells[10,20,36]. Theoretically the generation time of a cell would seem to be a constant, but this is not the case. In practice, cells grown under a variety of conditions and in different laboratories give different generation times[16]. A cell generation is the time taken for

a cell to traverse the cell cycle. This could be determined if the entire population of cells went into division at the same instant[25]. Unfortunately many problems occur from synchronizing techniques[15,16, 25,48,49,53,55,56,58]. Difficulty then occurs in the interpretation of such data. The major problems arising from the use of chemicals to synchronize cells are those involving biochemical agents such as colchicine, FUdR*, nitrogen mustard, vinblastine sulfate, and X-rays. These agents alter the cellular metabolism[15,20,48,49,55], and therefore affect the very processes under investigation. A survey of the literature would lead one to suspect that use of these drugs and agents can be valuable as long as reproducible results are obtained. Preliminary studies with colchicine, thymidine, and FUdR indicate the nucleic acid synthesis is drastically impaired. Thymidine and FUdR affect the kinetics of DNA synthesis. Colchicine was used in an attempt to obtain a greater yield in number of mitotic cells but with an unacceptably poor result. A satisfactory degree of synchrony was obtained without the use of drugs by either growing the cells to confluence or by employing selective detachment of mitotic cells[40,52].

The cell cycle can be visualized as a miniature life cycle that follows a definite sequence[34]. Throughout the cell cycle DNA, RNA, and protein are synthesized at specific times[51]. Beginning immediately

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*FUdR - 1-Fluoro-2-deoxyuridine

after mitosis the order of synthesis is protein (G_1), DNA (S), RNA (Late S), and finally a second group of proteins (G_2). The proteins synthesized before DNA are those needed for DNA synthesis. The first group of proteins are required for cell structure and are manufactured in G_1. DNA is of course, synthesized throughout the S phase while RNA occurs later in the same phase. The second group of proteins synthesized in G_2 are essential for subsequent DNA synthesis in the two daughter cells.

In order to study the above events synchrony was induced by two methods: 1) by simply shaking the mitotic cells off the glass (selective detachment of mitotic cells), and 2) growing the cells to confluency which stops their activity in G_1[37].

The objective of the study was to determine whether there were measurable biochemical differences at the cellular level between Down's syndrome fibroblasts and normal fibroblasts. Down's syndrome is characterized by a trisomy of G group chromosomes. The effect of this extra chromosome on DNA, RNA, and protein metabolism now preoccupies many workers. It was suggested by Mittwoch[35], and Kaback[25] that Down's syndrome cells spend more time in DNA synthesis. This observation has not been conclusively proven. By utilizing the two methods of cell synchrony as well as an asynchronous system this study provides quantitative information on the length of S phase in Down's syndrome and normal cells. Studies to be reported elsewhere have shown that Down's syndrome cells spend a minimum of five hours more than normals in synthesizing their genome. Before one can

elaborate on the process of DNA synthesis an important distinction must be made between a cell population doubling and a cell generation time.

In cell lines division proceeds continuously in the whole population and all the products of cell division survive. Therefore, the time taken to double their number is equivalent to one cell generation. On the other hand in cell strains a varying number of the cells in culture are generatively inactive at any given time and their period of mitotic competence is limited. Under these circumstances it can take considerably longer to double the population than it takes for active cells in the group to go through a single generation. Under ideal conditions cell strains go through a period of exponential growth and can almost double its population in one generation.

Cell strains are not guided by the same regulatory mechanisms as cell lines.

Strains are limited by contact inhibition of cell growth[6,28], a decreasing population of cells capable of division[21], a finite life span[21], and cell death[32]. Human diploid fibroblasts are cell strains, consequently the doubling time much exceeds a single generation span (generation time)[8].

Important factors in the regulation include changes in membrane structure, permeability, and charge. Once they have grown to confluence (where they are in direct contact with their neighbors) division ceases unless the cells are restimulated. In the majority

of cell lines contact inhibition occurs in a very limited degree if at all.

Cell strains suffer from marked density inhibition. Cells that have undergone contact inhibition can be regarded as having entered G_o. In this state there is loss of polysomes, deletion of endoplasmic cisternae and an increase in microfilaments[39]. The intracellular concentration of most enzymes increases ten-fold at confluency[9,13,39]. This is a reflection of the amount of protein being synthesized. Therefore, G_o really reflects a prolonged G_1. Release from this G_1 state can be initiated by adding serum to the medium or reversing confluence (trypsinization)[2,12,24,45,56,57]. This results in DNA synthesis 12 - 16 hours later, depending on the cells used. The serial cultivation of human diploid cell strains in this manner has been thoroughly reviewed by Hayflick[21].

A comparison of the human Down's syndrome fibroblasts and the normal fibroblast cultures wasundertaken in order to determine if there were discernable biochemical differences due to the presence of an extra chromosome 21. The literature contains many references to normal human diploid cells and their generation times determined asynchronously[10,20]. The Down's syndrome fibroblasts have not been intensely investigated.

The majority of work on Down's syndrome has been done at the level of translation (RNA - protein). Many serum proteins have been examined quantitatively as well as qualitatively: notably DNA

polymerase[17], lactate dehydrogenase, alkaline phosphatase[50], acid phosphatase[50], have exhibited demonstrable enzymatic differences. This approach has characterized the problem but has not elucidated the basic cause for the metabolic differences. In light of the above background facts, it appeared worthwhile to look into the following aspects of the problem.

I. Is there significant alteration in generation time in Down's syndrome.

A) What is the length of "S" phase?

B) What it the total length of generation time?

C) Could this system confirm a shorter G_1 for mongoloid cells?

II. What specific metabolic alterations are present in D.S.

A) D.N.A.

B) R.N.A.

C) protein

D) enzymes

III. To elucidate the length and causes of the apparent lag phase.

The above questions were approached in the following manner:

1. Fibroblasts were cultured from foreskins and skin biopsies.
2. Synchrony was induced:
    a) by selective detachment
    b) by growth to confluence
3. Continuous labelling experiments on both systems with tritiated thymidine.

4. Continuous labelling experiments with ^{14}C-leucine on both systems to examine protein synthesis.
5. The lag period was investigated with tritiated thymidine in the synchronous, and asynchronous system.
6. The total generation time of D.S. cells and normals were compared.

MATERIALS AND METHODS

A. Establishment of Primary Cultures:

This investigation was carried out with skin fibroblast cultures derived from foreskin samples or from punch biopsies of forearm skin. Six donors were utilized as the source of these cultures, and are designated either NF, indicating a normal donor, or MS, indicating a Down's syndrome donor. In all cases, the procedure for establishment of fibroblast cultures was as follows:

The skin samples (either foreskin or forearm) were obtained under sterile conditions. They were then washed in Hank's balanced salt solution and transferred to a petri dish with a small volume (5 ml.) of sterile Eagle's Minimal Essential Medium supplemented with 20% Fetal Calf Serum and antibiotics (50 µg, 50 units, and 50 µg respectively of Streptomycin, Penicillin, and Neomycin). The tissue was minced with sterile scissors to provide inocula of approximately 1 mm^2, and 10 - 15 such pieces were transferred to each sterile flask (Falcon T-30) using a Pasteur pipet. Additional MEM was added to give a volume of 1.5 ml/flask. The flasks were gassed with 5% CO_2-95% air, and placed in a water jacketed incubator. After 7 days, the medium was replaced with fresh MEM; this was repeated 7 days later. After 7 - 14 days, extensive zones of fibroblast cell growth could be observed around several of the explants in each flask. At this time the medium was removed, the flasks were rinsed twice with 0.25% Trypsin (1:250, Difco) and the flasks were returned to the incubator

for 10 minutes. The cells could then be dislodged by tapping the flask against the side of a hand, leaving the explants and epithelial outgrowth attached. The cells were then suspended in 3 ml. of MEM (20% serum), transferred to a new T-30 flask, gassed, and returned to the incubator. To minimize effects of residual trypsin, the medium was replaced the following day. This was considered to be the first passage.

When the flasks were about three-quarters confluent, the medium was replaced with MEM with 10% fetal calf serum and antibiotics as described above. The cultures were then left for 3 - 4 days to become confluent and accustomed to the reduced serum supplement. Thereafter the cultures were routinely passed by trypsinization as described above, and sub-cultured in the 10% MEM in such dilution as would give 1:10 confluence in the receiving flask. At this dilution the cells would grow to con-fluence in 8 - 10 days. Experiments were done using cells which had equal *in vitro* age and as far as possible were derived from age-matched donors. In the figures, the *in vitro* age (number of subcultures at 1:10 dilution) is indicated by a number following the NF or MS designation.

B. Synchronization by Selective Detachment:

Thirty glass bottles (150 $cm.^2$, Microbiological Associates) of each cell strain, age matched *in vitro*, not *in vivo*, in log phase were required to provide enough mitotic cells for each experiment. The bottles were agitated in an Eberbach mechanical shaker, excursion rate 16X/10 sec., and the supernatant fluid was removed and collected in ice. The detached cells were collected by centrifugation of the

total supernatant collection, at 900 rpm x 10 min., in the IEC PR-6 refrigerated centrifuge. The cell pellet was resuspended in a small known volume of medium, and aliquots were taken for determination of cell concentration and mitotic index. The cells were then diluted into the standard culture medium, containing ^{3}H-TDR (27 Ci/mM) at 0.2 µc/ml concentration, and the cell concentration adjusted to 10,000 - 12,000 cells/ml. Aliquots of 1.5 ml. were dispersed into sterile scintillation vials pre-conditioned for cell growth by three rounds of cell growth plus washing in tissue culture detergent (Micro-Solv, Microbiological Associates). Triplicate samples were provided for each time interval, for each cell strain used. These cultures were grown in a water jacketed CO_2 incubator with continuous flow of 95% air, 5% CO_2.

Cells were harvested at 3 hour intervals. To each vial was added 0.50 ml. of 0.5N NaOH to dissolve the cells. Five volumes of 7% TCA was added to precipitate the DNA and the acid insoluble material was collected by vacuum filtration on a glass fiber filter. The filter was washed 3 times with TCA and 3 times with 95% ethanol. The filter was then dried and added to 8 ml. scintillation fluid (0.1 g PPO, 4.12 g POPOP/liter in scintillation toluene), and counted for 5 minutes in a Packard Tri-Carb liquid scintillation system. The results were then plotted as shown in "RESULTS".

C. Synchronization by Confluence:

Cultures of D.S. and normal fibroblasts which were age matched *in vivo* and *in vitro*, were routinely passed at 1:10 dilution into glass bottles (150 cm.^{2} surface area) as described previously. The cultures were allowed to become confluent, and harvested at 11 days after subculture, at which time no mitotic cells had been observed for at least 2 days. The medium was not changed during this time. Cells were removed with trypsin, collected by centrifugation, and resuspended in a minimal amount of medium for cell number determination. The cells were then diluted to the required concentration (to give 10^4 cells/flask) in standard culture medium containing 1 µc/ml. ^{3}H-TDR (27 ci/mM), and aliquots were seeded into Falcon T-30 flasks. Duplicate cultures were provided for 2 hour sampling intervals to 28 hours post-harvest. At each sampling interval the medium was removed, the flasks were washed 1X in Hank's balanced salt solution, and the cultures were processed for scintillation counting as described in (B), above. The results are shown in Figure 1.

To determine the rate of total protein synthesis in this confluent system, cells were seeded into T-30 flasks, as above, in the same culture medium but with ^{14}C-leucine at a concentration of 0.1 µc/ml., as a tracer. The same sampling procedures described above were used to obtain precipitated protein for scintillation counting.

D. Asynchronous Pulse Chase:

A normal and mongoloid cell strain were grown to log phase on cover slips in Petri dishes placed in 5% CO_2 incubator; the medium was MEM supplemented by 10% FCS, and 50 µg/ml. Penn-Strep plus 50 µg/ml. Neomycin. Pulse labelling was achieved by exposing 54 hour cultures for 30 minutes to medium containing ^{3}H-TdR (Sigma Chemicals) (1 µc/ml.) (sp. act. 27 Ci/mM). The ^{3}H medium was removed and the cells were washed 3 times with MEM kept at 37°C. Fresh 10% FCS MEM was added. No excess cold thymidine was added. Samples were removed every 2 or 3 hours and fixed in 3:1 methanol-acetic acid. The slides were dipped in Kodak NTB^2 emulsion and then left in scintillation toluene, containing 0.1 g PPO and 4.12 g POPOP/liter. The slides were then carefully washed overnight in cold H_2O and stained in cresyl violet. The number of labelled and unlabelled metaphases, anaphases and telophases was determined for 50 mitoses per sample. The period where 90-100% of mitosis were labelled was considered the "S" period.

RESULTS

1. Uptake of ^{3}H-TdR after confluence release:

The data in Figure 1 and Figure 2 was obtained from D.S. and normal cells grown to confluence, then subcultured to allow exponential growth. After release from confluence and subsequent cell growth, both Figure 1 and Figure 2 show that there is a 12 to 16 hour lag in both D.S. and normal cells before the start of DNA synthesis. This is the period required for the cell to attach, repair any damage that may have occurred in the subculture process, and to complete G_1.

Increase in ^{3}H-TdR uptake in Figure 2 starts in both D.S. and normal cells at 12 hours after subculture. The completion of S, indicated by a change in slope and levelling off of ^{3}H-TdR incorporation, occurs between 22 and 24 hours for normal cells and at 26 hours for D.S. cells. The S period for normal cells would be 10 to 12 hours and for D.S. cells 14 hours. The uptake of ^{3}H-TdR for normal cells in Figure 1 shows a sharp increase 16 hours after subculture and levels off at 24 hours. In this experiment data shows that the minimal time for S period for control cells is 8 hours. D.S. cells increased their incorporation of ^{3}H-TdR at 16 hours. The increase in radioactivity had not levelled off when the experiment was terminated at 28 hours. In this experiment the minimal time for S would be 12 hours or 4 hours greater in D.S. than normal cells.

Growth to confluence arrests cells at G_1 boundary and release from this allows cells to complete G_1 and proceed into S. The synchrony of the cell cycle obtained is usually not as complete as that obtained by selective detachment. Normal cells in Figure 2 had a plateau for ^{3}H-TdR incorporation which extended from 24 to 32 hours. During this period the cell progresses through G_2, M and G_1. The sharp increase in the slope at 32 hours could indicate the beginning of another S period. This would give a cell replication time of 20 hours equally divided between S and other phases of the cell cycle. The increase in incorporation could be due to cells out of phase but with the magnitude of the increase this is likely not to be the case. Down's syndrome cells did not complete S until between 24 and 28 hours. The period to complete G_2, M and G_1 appears shorter in D.S., 4 to 6 hours, than in normal cells. As G_2 and M take a minimum of 4 hours[12,14], the period in G_1 is shorter in D.S. than normal cells.

2. Protein content and synthesis after confluence release:

Incorporation of ^{14}C-leucine into cell protein and actual amount of protein/10^6 cells is shown in Figure 3. Down's syndrome and normal cells show a similar pattern of incorporation of ^{14}C-leucine/10^6 cells at all periods studied from 4 through to 32 hours. From 4 to 8 hours after confluence release protein synthesis occurs as shown by increase in ^{14}C-leucine incorporation. At 12 hours in both cultures, there is a drop in ^{14}C incorporation as well as an actual decrease in cell content of protein. This is a consistent observation seen in other

experiments. A marked increase in synthesis of protein occurred between 12 and 16 hours and continued through the study period of 32 hours. A marked increase also occurred in protein content of the cells between 14 and 16 hours when this phase of the study was terminated. The increase in protein content and synthesis coincided with DNA synthesis occurring in the S period in both types of cells.

3. Determination of the lag period in normal and D.S. fibroblasts:

It has been observed that when cells are subcultured, a period of 14 - 16 hours occurs before DNA synthesis occurs. This time is thought to be the time necessary for the cell to repair any damage that has occurred in the subculture process, to adjust pool sizes of various metabolites and to prepare to enter S. To differentiate the length of time for this transition, cells derived from the same donor and of equal *in vitro* life span were used. Cells that were growing exponentially were used to determine the uptake of ^{3}H-TdR in log phase cultures. Cells that had been grown to confluence then released, were used to determine the lag period for ^{3}H-TdR uptake. The results showed that when the first sample was taken from log phase cultures at 5 hours, there was a similar but significant uptake of ^{3}H-TdR in normal and D.S. cells. There was no significant uptake at this time in either cell line from confluent released cultures. At 10 hours, there was a larger increase in retained radioactivity as expected in exponentially growing cells that was similar for D.S. and normal cell lines. At 15 hours

confluence released cells began to incorporate ^{3}H-TdR which was greatly increased at 24 hours for both normal and D.S. cells. The confluence released cultures that had the same number of cells, took 24 hours to reach the same level of ^{3}H-TdR incorporation as the exponential cells reached at 16 hours. The lag period defined in this manner is 8 - 10 hours in both D.S. and normal cells.

DISCUSSION

The process of DNA synthesis is one of the most important and fundamental steps to a cell's subsequent integrity. Since the D.S. cells have an extra chromosome it is reasonable that these fibroblasts may take longer to synthesize their genome if the chromosome is late replicating. Kaback[25] and Mittwoch[35] have measured the DNA content of non-dividing nuclei in asynchronous cultures of mongoloid cells. From their studies they have implied that the rate of DNA synthesis is slower in trisomic cultures. The present work shows a longer S period in D.S. cells. Synchrony studies have shown this difference to be of the order of 2 to 4 hours *in vitro*. This change must be suspected of altering the generation time of these cells. We have found (Segal and McCoy) that the doubling time from growth curves shows a significant difference between D.S. cells, and age-matched controls. Defendi[10] and others have stated that the length of the generation time[36] found in various cell populations varies considerably depending on the function and conditions of cells. In all cases the fibroblast cultures were maintained in controlled environments as well as matching them *in vitro* and *in vivo*. The experiments on the length of S demonstrated that the methods of synchrony induction and precursor uptake in both cell types gives a reliable comparison of the length of DNA synthesis. In the systems used, DNA synthesis took 10 - 12 hours in normal cultures and 12 - 14 hours in D.S. cells. Thirteen hours is the more realistic

figure for D.S. since the experiments with the confluent system of synchrony utilized age matched cells. The lengthening of DNA synthesis may be a result of late replicating chromosomes[36].

Maceira[30,31] has shown that the increase in generation time is due to changes of G_1 and G_2. G_1 and G_2 are gaps which can be changed by the cell in adapting to a new environment. This environmental change may be serum addition[2,12,24,45], changes in pool size[41,57], or trypsinization[5]. The changes induced by release from contact inhibition and subsequent serum stimulation allow the cells to enter a phase of proliferation and growth. In the experiments the release from the G_o (G_1 state) allows a majority of cells to enter DNA synthesis 15 - 17 hours later[21,37,38,39]. This holds true for both cell types.

The length of G_1 varies from established cell lines to normal fibroblast strains. G_1 also varies according to the method of analysis. If measured by continuous labelling after synchrony by selective detachment M and G_1 shows a length of 15 - 17 hours. This period of time no doubt includes the lag time for these cells. This lag is interpreted to be the length of time taken by the cell to become metabolically active. These lag times are absent or are much more subtle in cells which do not demonstrate contact inhibition[26]. This lag is also absent when the cell cycle is studied in an asynchronous system. This may be due to two factors. First, the cells are grown for 24 hours to avoid synchrony. Second, the cells being asynchronous cannot demonstrate the lag to the same degree as in the synchronous

system. Cell doubling time for cell lines is synchronous with generation time. This does not hold for human diploid fibroblasts or 3T3 cells[37] which show a marked contact inhibition state. Irrespective of the mode of synchrony, 15 - 18 hours was the time taken by cells to begin DNA synthesis. This time likely represents in part, at least (since synchrony was invoked at different states of the cell cycle), an adjustment period. Hayflick[21] has shown that the proportion of diploid cells failing to divide when they are subcultured increases with age in the culture. This reflects the inability of certain cells to make preparative adjustments for continuous metabolic activity. The major differences in kinetics of the cell cycle between normal and trisomic cultures are the increased length of S in D.S. cells and the increased length of G_1 in normal cells.

The cell cycle differences give D.S. cells a minimum generation time of 18.5 hours, while normal cells have a 13.5 - 14.5 hour generation time. The time taken for normal cells to traverse G_2 + M + G_1 is equal to 8 hours. D.S. fibroblasts take 6 hours to traverse the same phases of the cell cycle. Unpublished data from this laboratory on RNA/DNA ratios as well as protein/DNA ratios in logarithmic cultures of normal and D.S. cells showed results similar to those reported by Kaback[25].

In Figure 3, which shows the rate of ^{14}C-leucine incorporation, it was determined that after confluency release there was significant variation in the rate of protein synthesis in both trisomic and normal fibroblasts. There is a significant decrease evident in amount of

protein per cell 10½ hours after release from confluence. Analysis of protein/10^6 cells as well as rate of ^{14}C-leucine incorporation demonstrated in Figure 3, that at a period of 10 - 12 hours after release from confluence there is a significant drop in protein per cell. Since there are specific periods when proteins are synthesized in a synchronous system, it is possible that this decrease reflects a change in the amount of soluble protein present per cell. Protein determination and ^{14}C incorporation both demonstrate these dramatic changes. This information added to the sequential steps which are already known to occur in the cell cycle are sufficient proof that the cells go through a necessary programmed series of biochemical events. The time of this initial burst of protein synthesis and then subsequent degradation needs further analysis. Notably the time occurrence in both normal and D.S. fibroblasts are identical. The importance of protein synthesis and DNA synthesis are well established. The occurrence of this additional peak of protein synthesis has also been observed in a Chinese hamster cell line[7]. This synthesis of protein then does not seem to be solely a property of G_o cells. Investigations are continuing to determine further biochemical differences between D.S. and normal fibroblasts.

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FIGURE 1

RATES OF ^{3}H-THYMIDINE INCORPORATION INTO DNA AFTER SYNCHRONY BY CONFLUENCE IN D.S. AND NORMALS

●—● represent the normal and ▲—▲ represent the mongoloid cells plotted at 4 hour intervals. Cell number was monitored throughout the experiment. Cells confluent after 11 days.

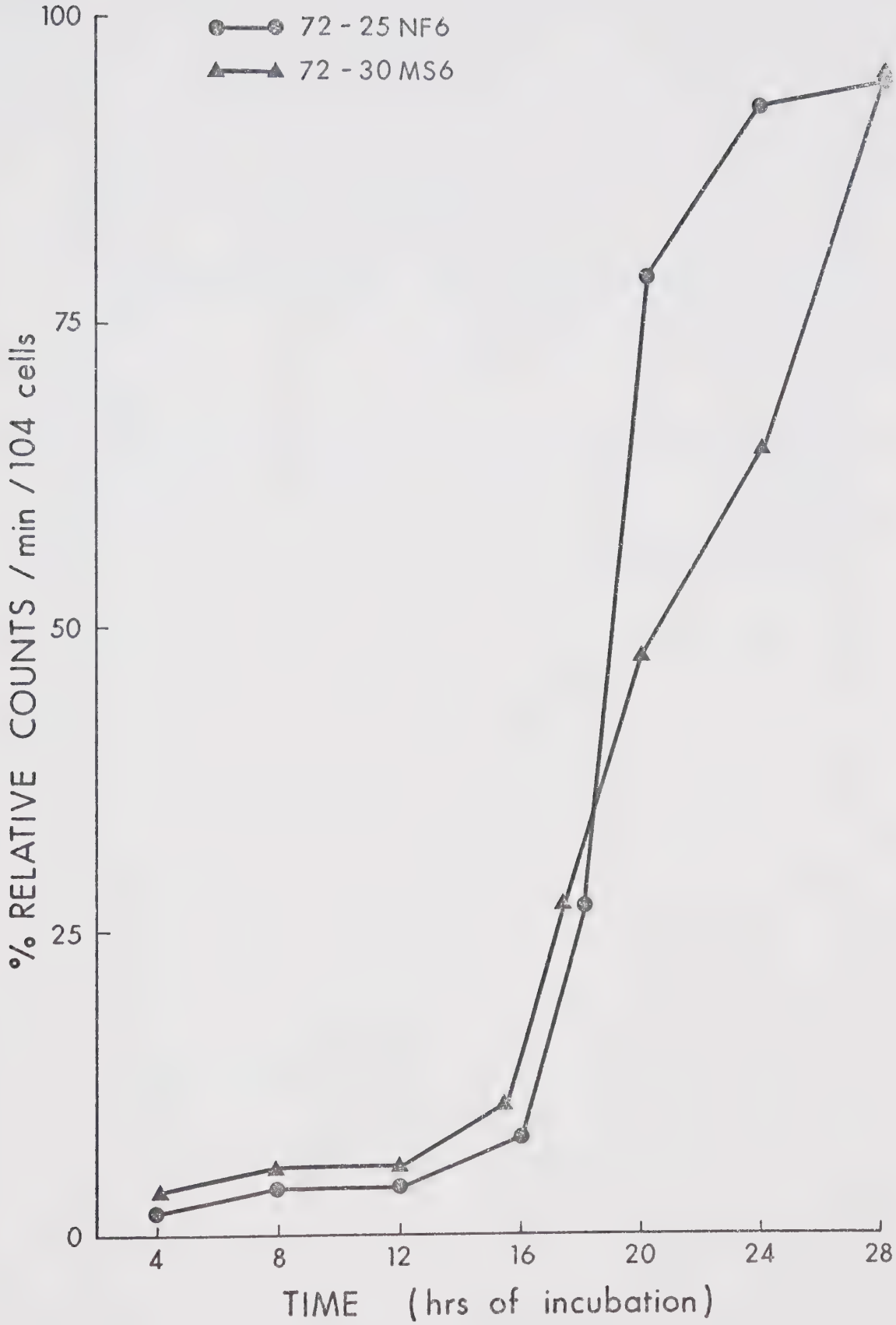

72 - 25 NF6
72 - 30 MS6
% RELATIVE COUNTS / min / 104 cells
100
75
50
25
0
4
8
12
16
20
24
28
TIME (hrs of incubation)

FIGURE 2

RATES OF ^{3}H-THYMIDINE INCORPORATION INTO DNA AFTER SYNCHRONY BY CONFLUENCE IN D.S. AND NORMALS

●—● represent the normal and ▲—▲ represent the mongoloid cells plotted at 4 hour intervals. Cell number was monitored throughout the experiment. Cells confluent after 8 days.

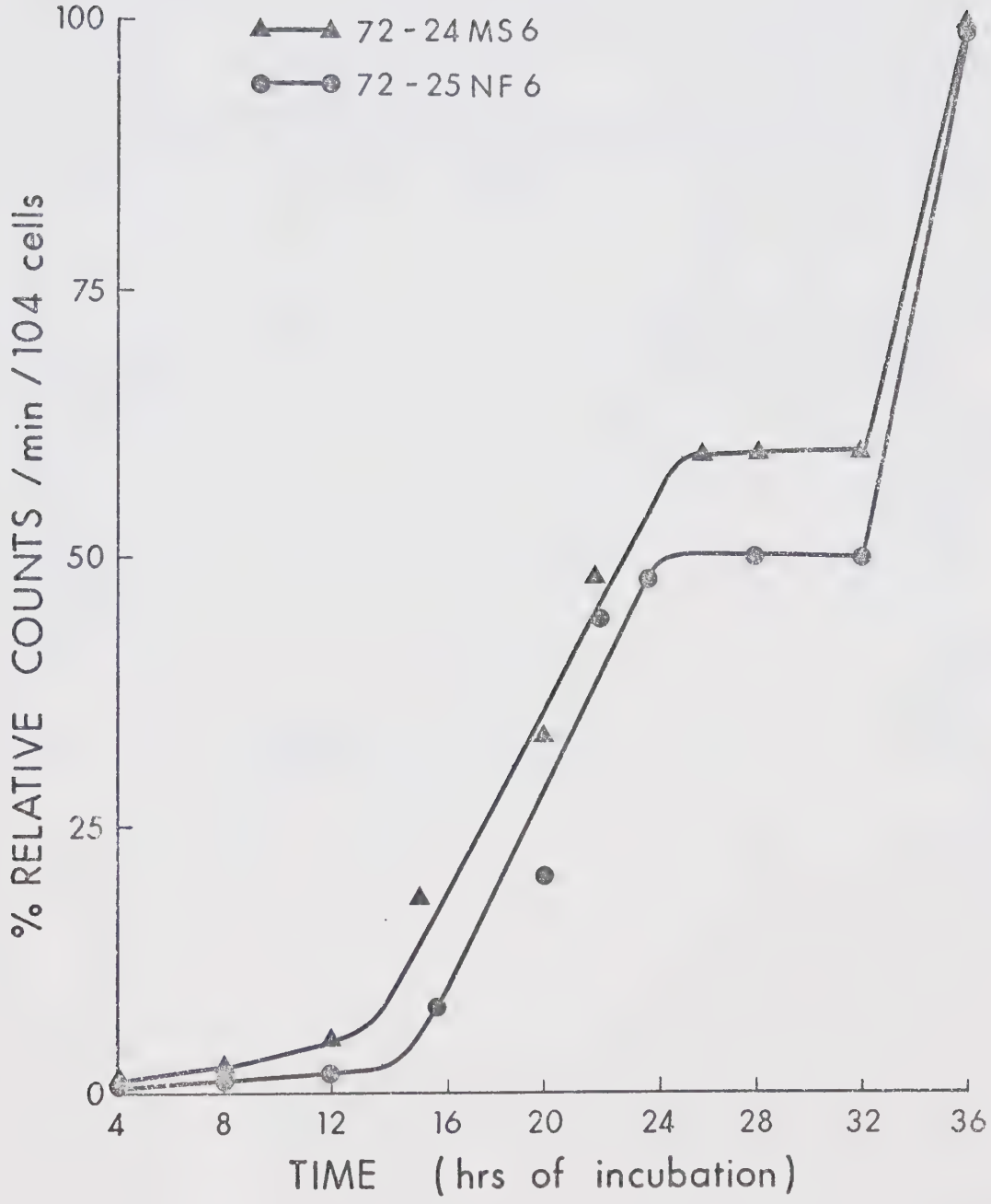

72 - 24 MS 6
72 - 25 NF 6
% RELATIVE COUNTS / min / 104 cells
100
75
50
25
0
4
8
12
16
20
24
28
32
36
TIME (hrs of incubation)

FIGURE 3

PROTEIN SYNTHESIS IN SYNCHRONIZED MONGOLOID AND NORMAL FIBROBLASTS

The notations ●—● and o--o represent the profiles for ^{14}C-leucine incorporation in the normal strains 72-25NF8. The notations ▲—▲ and Δ--Δ represent the profiles for ^{14}C-leucine incorporation in the mongoloid strains 72-24MS8 and 72-5MS8, plotted at the same time interval. Cell number was monitored.

**Note:* On the graph, 72-5NF8 should read 72-5MS8.

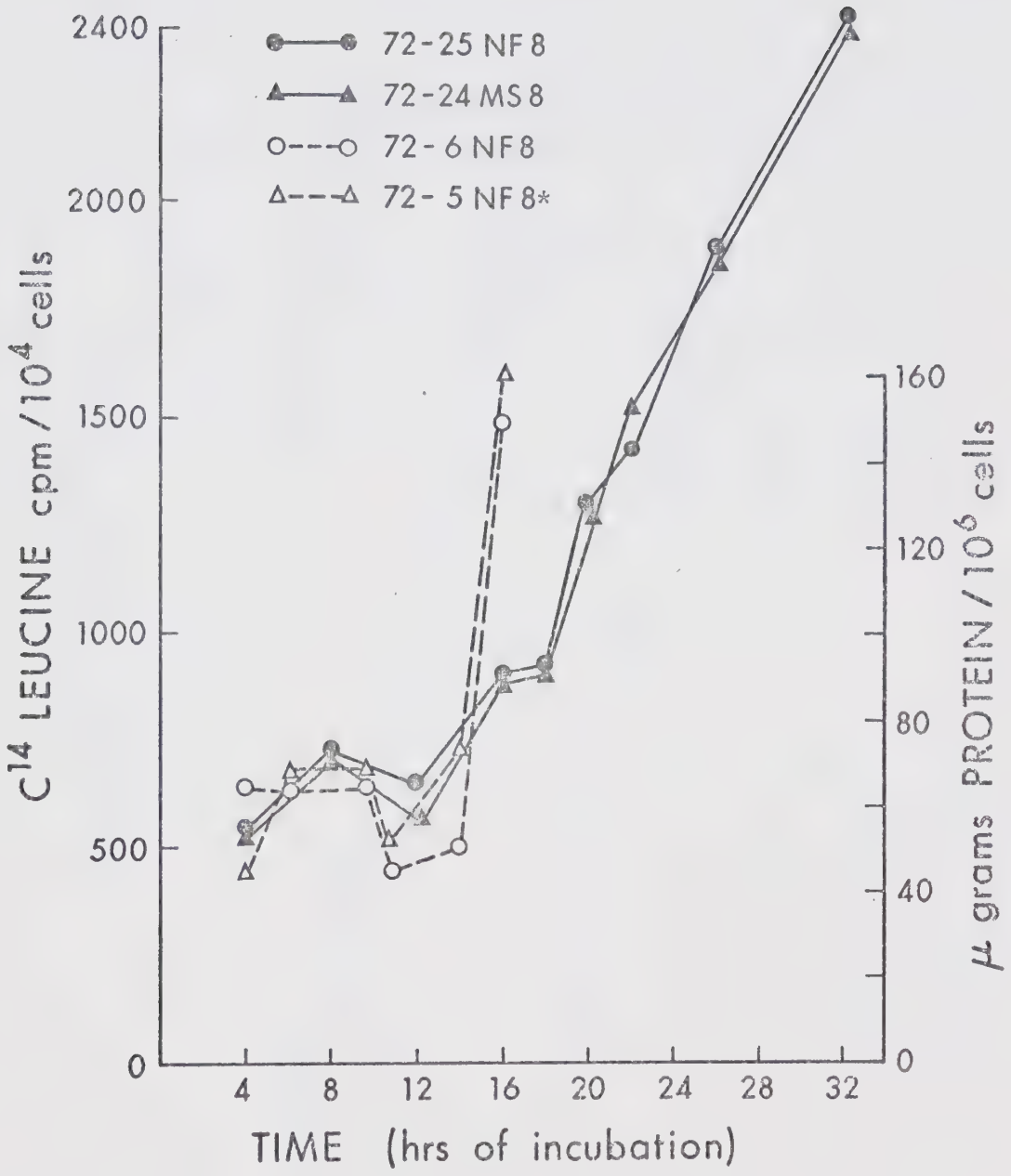

72-25 NF 8
72-24 MS 8
72-6 NF 8
72-5 NF 8*
C14 LEUCINE cpm / 10^4 cells
μ grams PROTEIN / 10^6 cells
TIME (hrs of incubation)
2400
2000
1500
1000
500
0
160
120
80
40
0
4
8
12
16
20
24
28
32

FIGURE 4

DETERMINATION OF LAG TIME IN D.S. CULTURES

▲—▲, ●—●, ■—■, are the log phase profiles for ^{3}H-TdR incorporation in lines 72-3MS8, 72-4MS8 and 72-5MS8. △—△, o—o, □—□, represent the same cultures grown under lag phase conditions. Percent relative counts/minute/10^{4} cells are defined under Figure 2.

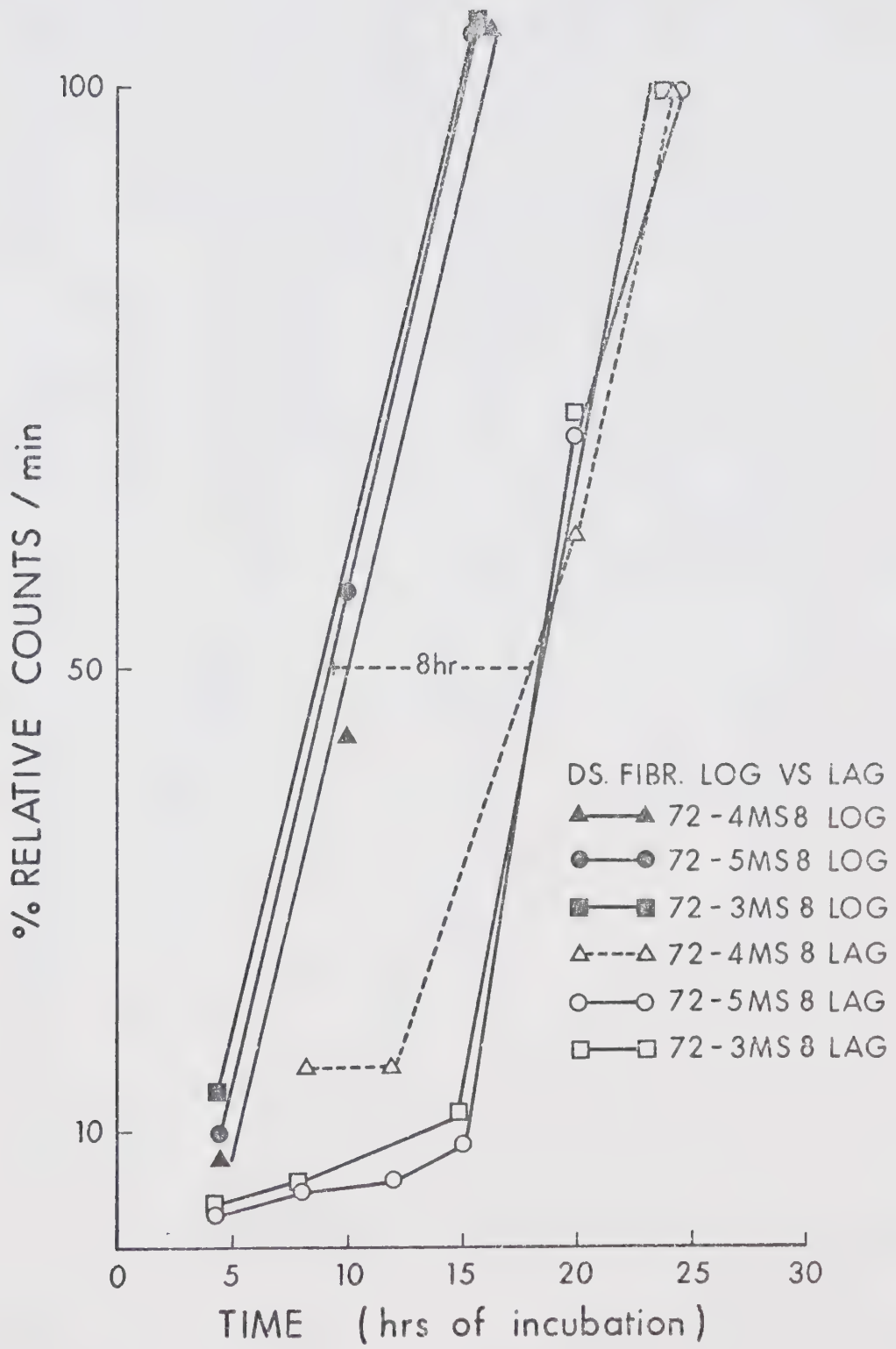

100
50
10
% RELATIVE COUNTS / min
8hr
DS. FIBR. LOG VS LAG
72 - 4MS 8 LOG
72 - 5MS 8 LOG
72 - 3MS 8 LOG
72 - 4MS 8 LAG
72 - 5MS 8 LAG
72 - 3MS 8 LAG
0
5
10
15
20
25
30
TIME (hrs of incubation)

FIGURE 5

DETERMINATION OF LAG TIME IN NORMAL CULTURES

Cells used in this experiment are presented on the top right corner of Figure 5. Lag and log cultures are defined by appropriate figures. Cell number was monitored as in Figure 4. ^{3}H-TdR incorporation is represented in the same manner as in Figures 1, 2, and 4.

**Note:* on the graph 72-17NF8 should read 71-17NF8.

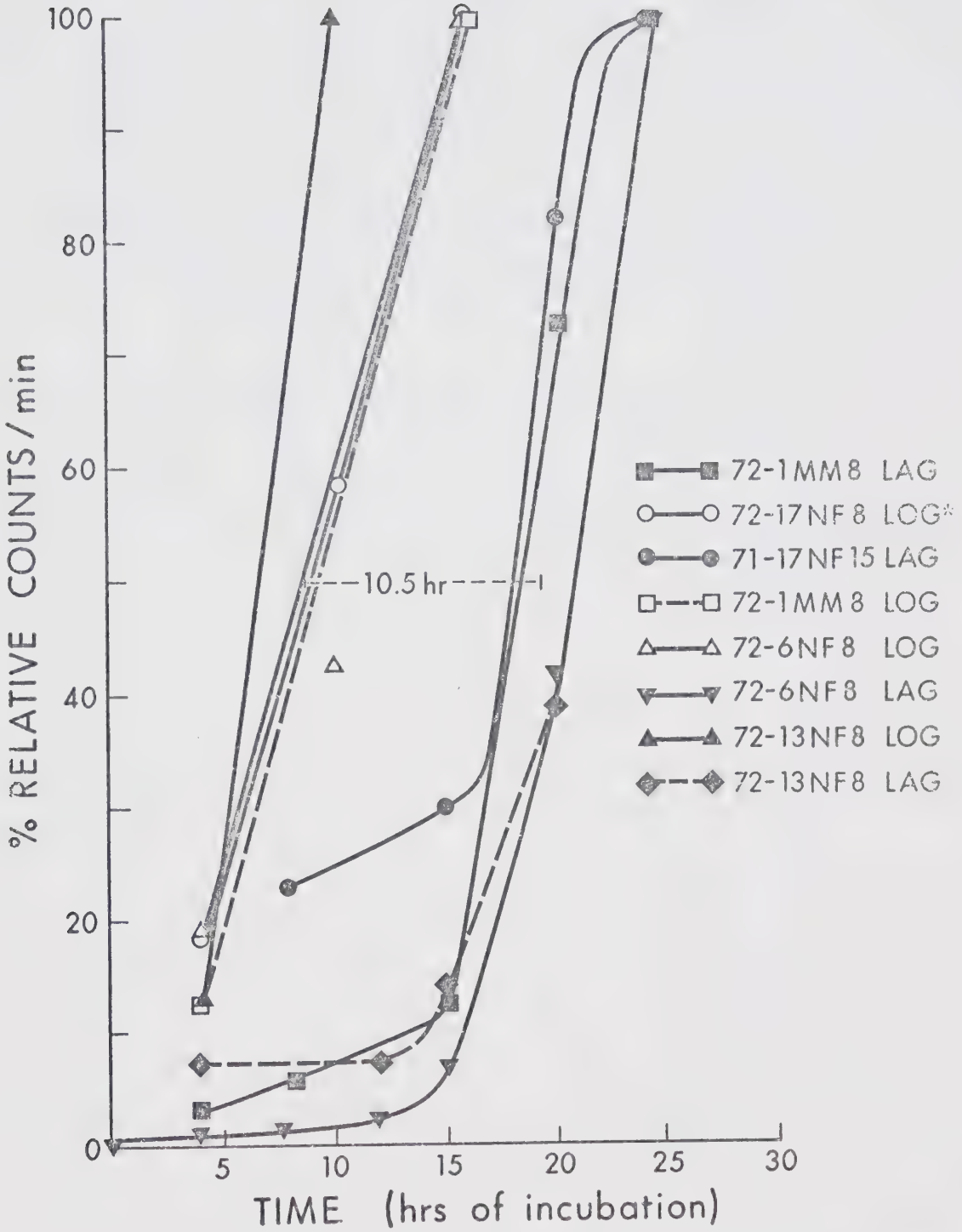

100
80
60
40
20
0
% RELATIVE COUNTS / min
10.5 hr
5
10
15
20
25
30
TIME (hrs of incubation)
72-1MM8 LAG
72-17NF8 LOG*
71-17NF15 LAG
72-1MM8 LOG
72-6NF8 LOG
72-6NF8 LAG
72-13NF8 LOG
72-13NF8 LAG

FIGURE 6

^{3}H-THYMIDINE INCORPORATION INTO DNA AFTER SYNCHRONY BY SELECTIVE DETACHMENT IN D.S. AND NORMALS

▲—▲ represent normal cells (72-6NF) and ●—● represent the mongoloid cells (72-5MS). Cell number was monitored; however, the normals had approximately two times as many cells as mongoloids.

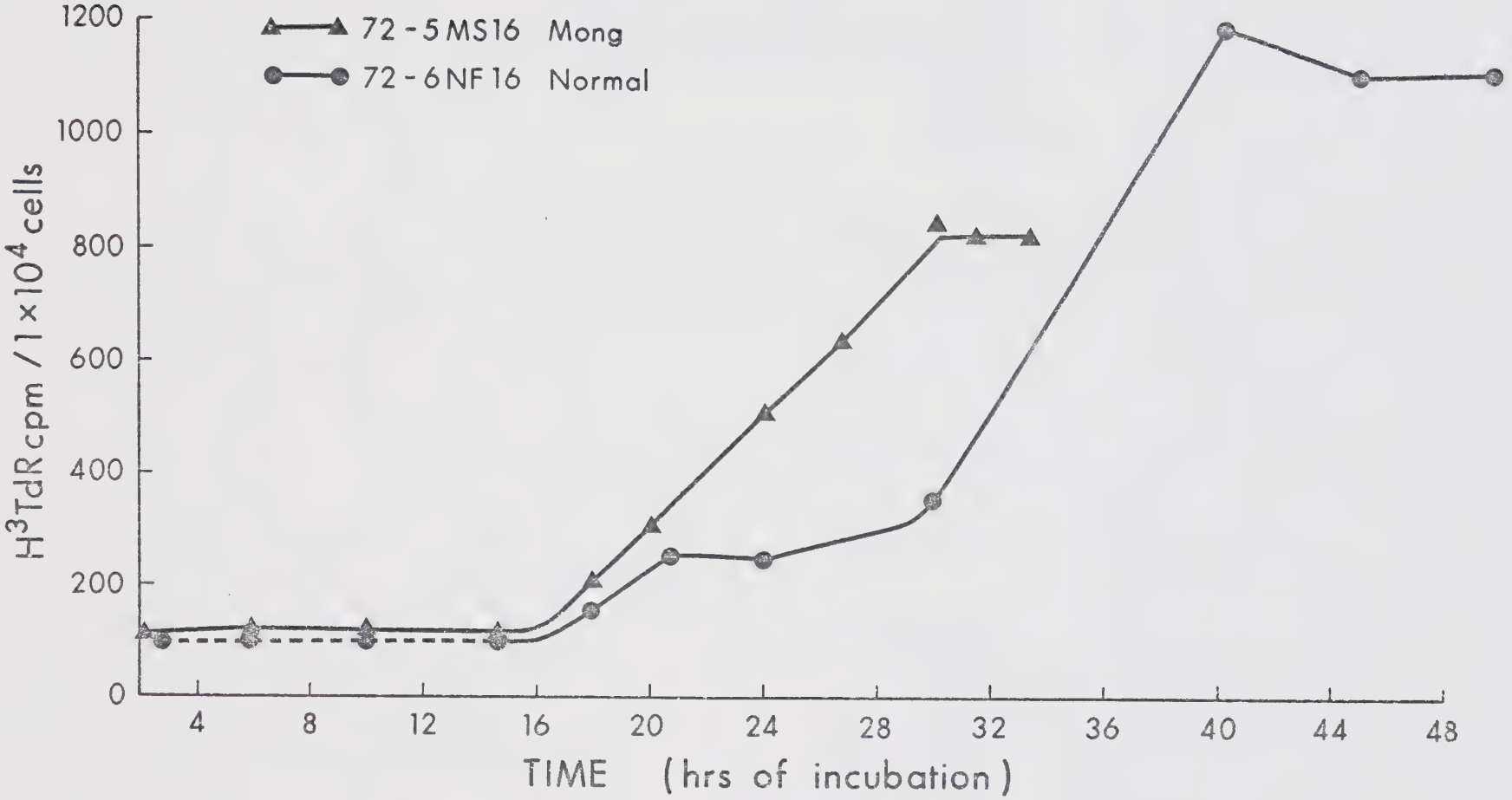

72 - 5 MS16 Mong
72 - 6 NF 16 Normal
H^3TdR cpm / 1×10^4 cells
1200
1000
800
600
400
200
0
4
8
12
16
20
24
28
32
36
40
44
48
TIME (hrs of incubation)

FIGURE 7

^{3}H-THYMIDINE INCORPORATION INTO DNA AFTER SYNCHRONY BY SELECTIVE DETACHMENT IN D.S. AND NORMALS

▲—▲ represent the mongoloid cells (72-5MS8) and ●—● represent the normal cells (72-6NF8). Cell number was equal in both D.S. and controls.

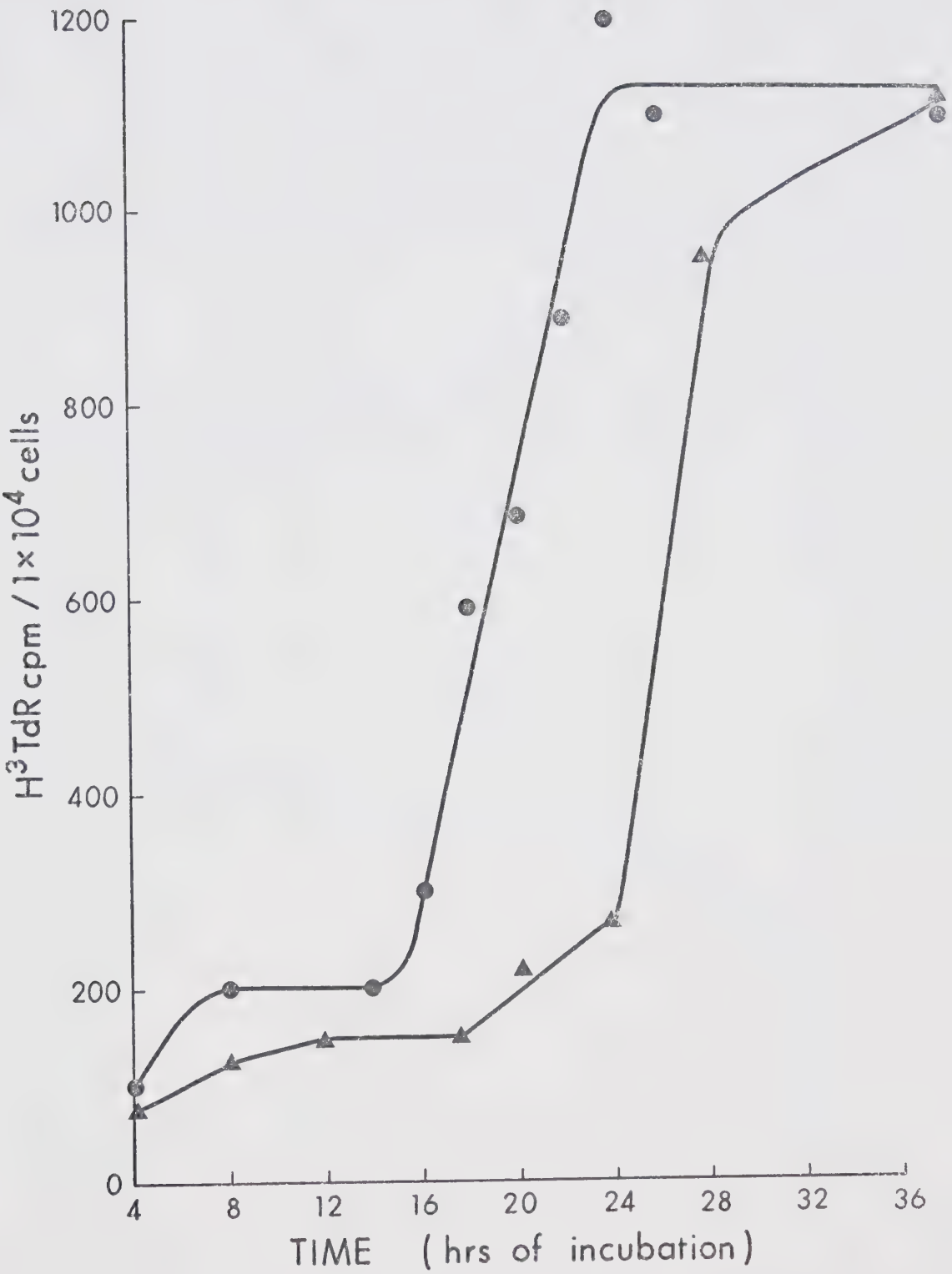
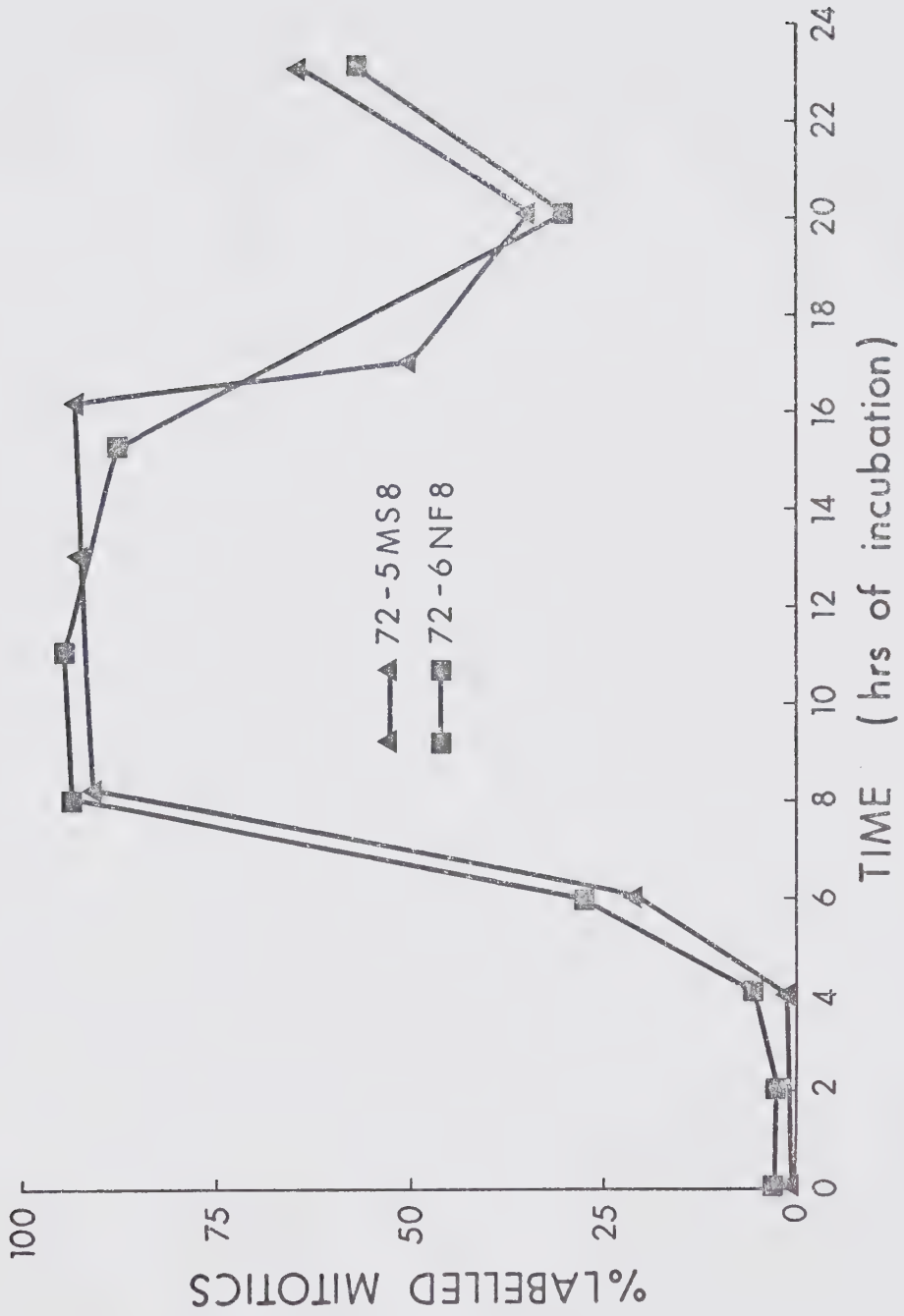

1200
1000
800
600
400
200
0
H^3TdR cpm / 1×10^4 cells
4
8
12
16
20
24
28
32
36
TIME (hrs of incubation)

FIGURE 8

ASYNCHRONOUS PULSE-LABELLED ^{3}H-THYMIDINE INCORPORATION IN MONGOLOID *vs.* NORMALS

▲—▲ represent the mongoloids (72-12MS) plotted at two hour intervals. Solid line, ■—■, represents the normals (72-13NF). Each point represents a count of at least 50 mitotic cells per slide.


% LABELLED MITOTICS
100
75
50
25
0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (hrs of incubation)
72-5MS8
72-6NF8